

D²
containing this common 5.8 kb fragment was deposited in the National Institute of Bioscience and Human Technology, Agency of Industrial Science and Technology in accordance with the Budapest Treaty under the accession No. FERM BP-6916. Its microbiological characteristics were identical to those of E.coli HB101 except that it can degrade aromatic compounds and chlorinated aliphatic hydrocarbon compounds.--

IN THE CLAIMS:

Kindly amend claims 2, 3, 4, 9, 10, 11, 15, 17 and 55 as follows. A marked-up version of claims 2-4, 9-11, 15, 17 and 55 has been attached showing the changes made to the claims.

--2. (Twice Amended) A DNA fragment isolated from Burkholderia cepacia KK01 wherein the DNA fragment has a nucleotide sequence of SEQ ID NO: 1.

D³ Sub F²
3. (Twice Amended) An isolated DNA fragment having a nucleotide sequence that hybridizes under stringent conditions to a hybridization probe with a nucleotide sequence consisting of SEQ ID NO: 1 or a complement of SEQ ID NO: 1.

4. (Amended) A recombinant DNA comprising a vector enabling maintenance or replication in a host, said vector including a DNA fragment according to any one of claims 1 to 3.

Sub E1

9. (Twice Amended) An isolated DNA fragment containing a region encoding a toluene monooxygenase, wherein the region comprises a first sequence that hybridizes under stringent conditions to a hybridization probe of which nucleotide sequence consists of 463..1455 portion of SEQ ID NO: 1 or a complement thereof, encoding a polypeptide TomL having an amino acid sequence of SEQ ID NO:3 or a mutant thereof, a second sequence that hybridizes under stringent conditions to a hybridization probe of which nucleotide sequence consists of 1495..1761 portion of SEQ ID NO: 1 or a complement thereof, encoding a polypeptide TomM having an amino acid sequence of SEQ ID NO: 4 or a mutant thereof, a third sequence that hybridizes under stringent conditions to a hybridization probe of which nucleotide sequence consists of 1803..3350 portion of SEQ ID NO: 1 or a complement thereof, encoding a polypeptide TomN having an amino acid of SEQ ID NO: 5 or a mutant thereof, a fourth sequence that hybridizes under stringent conditions to a hybridization probe of which nucleotide sequence consists of 3428..3781 portion of SEQ ID NO: 1 or a complement thereof, encoding a polypeptide TomO having an amino acid sequence of SEQ ID NO: 6 or a mutant thereof, and a fifth sequence that hybridizes under stringent conditions to a hybridization probe of which nucleotide sequence consists of 3810..4871 portion of SEQ ID NO: 1 or a complement thereof, encoding a polypeptide TomP having an amino acid sequence of SEQ ID NO: 7 or a mutant thereof, and the first to fifth sequences are aligned so that expressed polypeptides can form an active monooxygenase protein.

10. (Twice amended) An isolated DNA fragment comprising a region that hybridizes under stringent conditions to a hybridization probe of which nucleotide

Ant E1
sequence consists of 234...443 portion of SEQ ID NO: 1 or a complement thereof,
encoding a polypeptide TomK having an amino acid sequence of SEQ ID NO: 2 or a
mutant thereof.

D4
11. (Three Times Amended) A recombinant DNA comprising a vector,
wherein said vector contains a promoter which is functionally ligated to a DNA fragment
according to any one of claims 6, 7 or 9, to enable expression of the toluene
monooxygenase encoded by the DNA fragment.

DS
15. (Twice Amended) A transformant obtained by introducing a
recombinant DNA into a host microorganism, the recombinant DNA comprising a vector
enabling maintenance or replication in a host and a DNA fragment of about 5.8 Kb
containing a toluene monooxygenase gene having 1 BamHI restriction site, 2 EcoRI
restriction sites, 1 HpaI restriction site, 1 KpnI restriction site, 1 NcoI restriction site, 1
NspV restriction site, 1 SacI restriction site, 2 SmaI restriction sites, 3 SphI restriction
sites, 2 XhoI restriction sites, no ClaI restriction site, no DraI restriction site, no EcoRV
restriction site, no HindIII restriction site, no NdeI restriction site, no NheI restriction site,
no PvuII restriction site, no ScaI restriction site, no Sse8387I restriction site, no StuI
restriction site, and no XbaI restriction site, said DNA-fragment derived from Burkholderia
cepacia KK01.

Sub E2
D6

17. (Twice Amended) A transformant obtained by introducing a recombinant DNA into a host microorganism, where the recombinant DNA comprises a vector enabling maintenance or replication in a host, said vector including a DNA fragment ligated thereto having a sequence that hybridizes under stringent conditions to a hybridization probe of which nucleotide sequence consists of SEQ ID NO: 1 or a complement of SEQ ID NO: 1 and encoding an active toluene monooxygenase, wherein the DNA fragment contains a toluene monooxygenase region of 4.9 kb or less.

Sub E2
D7

55. (Three Times Amended) A recombinant DNA comprising a vector, a promoter, a first DNA fragment being the DNA fragment of any one of claims 6, 7 or 9, and a second DNA fragment, said second DNA fragment comprising a region encoding a polypeptide TomK having an amino acid sequence of SEQ ID NO: 2 and a property to enhance the toluene monooxygenase activity of a protein comprised of at least TomL to TomP; or a region that hybridizes under stringent conditions to a hybridization probe of which nucleotide sequence consists of 463..1455 portion of SEQ ID NO: 1 or a complement thereof, encoding TomK or an active mutant thereof, wherein the first DNA fragment containing a toluene monooxygenase encoding region of 4.9 kb or less is functionally connected to the promoter to express an active toluene monooxygenase, and the second DNA fragment is functionally connected to the promoter to express a property to enhance the toluene monooxygenase activity.--